ISSN (print) 0093-4666 © 2010. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

DOI: 10.5248/113.405

Volume 113, pp. 405–413 *July–September 2010*

Phialophora sessilis, a species causing flyspeck signs on bamboo in China

Jieli Zhuang[1], Mingqi Zhu[1], Rong Zhang[1], Hui Yin[1], Yaping Lei[1], Guangyu Sun[1*], Mark L. Gleason[2]

sgy@nwsuaf.edu.cn

[1] *College of Plant Protection & Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A&F University, Yangling, Shaanxi, 712100, China*

[2] *Department of Plant Pathology, Iowa State University Ames, Iowa 50011, U.S.A*

Abstract —*Phialophora sessilis* is reported and redescribed from China. It is distinguished from the other known species in the genus by reduced, flaring phialidic collarettes and clusters of single-celled conidia. ITS sequence analysis of four strains from Xianning, Hubei, China, attributed to the species shows it to be clearly distinct.

Key words —phialide, taxonomy, genetic analysis, sooty blotch, *Gramineae*

Introduction

The genus *Phialophora,* which was introduced by Medlar for *P. verrucosa* Medlar isolated from a human skin lesion (Medlar 1915), is currently regarded as a member of *Herpotrichiellaceae* (Haase et al. 1999). It is a little-differentiated genus of more or less pigmented, phialidic hyphomycetes (Hoog et al. 2000). With the addition of numerous species, the genus has become grossly polyphyletic, although some taxa have already been segregated from *Phialophora* into *Cadophora* (*Helotiales*), *Harpophora* (*Magnaporthaceae*), *Lecythophora* (*Coniochaetaceae*) and *Phaeoacremonium* (*Togniniaceae*) (Kirk et al. 2008).

Most *Phialophora* species are common saprobes in soil, wood pulp, and other plant material. Others are more specialized plant pathogens, and human pathogenicity is known for a few species (Gams 2000). *Phialophora sessilis* was first reported by Hoog et al. (1999) from *Picea abies* resin in the Netherlands

*Corresponding author.

and described in a comparative study of 34 strains belonging to the *Phialophora verrucosa* complex. Additional strains of *Ph. sessilis* originated from forest soils in Sweden, the lichen *Peltigera polydactyla* (Hoog et al. 1999), and marble powder in Italy (Caretta et al. 2006). Important phenetic characteristics of *Ph. sessilis* are dark, slow growing colonies, conspicuous collarettes that are darker than the rest of the phialide and inserted laterally on undifferentiated hyphae, and conidia sometimes inflating and then frequently bearing phialidic collarettes (Caretta et al. 2006).

During a recent survey of host plants for flyspeck fungi in China, two bamboo species were found to be hosts of flyspeck related with *Phialophora sessilis*.

Materials and methods

Fungal strains

Four strains were isolated from the culms of two different hosts; isolates ZJ81-D5 and ZJ81-D7 were from *Phyllostachys meyeri*, and ZJ88-B3 and ZJ88-B8 were from *Yushania falcatiaurita*. Representative dried culture and plant specimens were deposited in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

Isolates

Individual sclerotium-like bodies (Batzer et al. 2005), growing in clusters on bamboo culms, were transferred to slants containing potato dextrose agar (200 g peeled potato, 20 g dextrose, 10 g agar in 1 L water; PDA) and cultured at 22 ± 1°C in the dark (Sun et al. 2003). Axenic cultures from slants were transferred to new PDA plates, a sterile cover slip was partially inserted into the agar adjacent to the colony and angled away from the colony at approximately 60 degrees to the agar surface in order to enable the fungus to grow onto the cover slip. Measurements of fungal structures were conducted based on isolates growing on cover slips. Colony descriptions were made after 1 month of growth on PDA plates at 22 ± 1°C in darkness.

DNA extraction, PCR, and sequencing

The protocol of Barnes et al. (2001) was followed to extract genomic DNA from fungal mycelium growing on PDA slants. The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify part of the nuclear ribosomal RNA (nrRNA) operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene. The PCR reaction mixture, consisting of 1 unit Taq polymerase, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, and 2 μL template DNA, was made up to a total volume of 25 μL with sterile water. Reactions were performed on a Bio-Rad PCR System PTC-200TM and the cycling conditions were an initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, and a final 10-minute extension step at 72°C. Purifying and automated sequencing with the

primer ITS4/ ITS1-F of the PCR product was performed at Organism Technology Co., Shanghai, China.

Sequence alignment and phylogenetic analyses

The ITS nucleotide sequences generated in this study were added to sequences of six species of *Phialophora* obtained from GenBank (www.ncbi.nlm.nih.gov) (Table 1). After importing into BioEdit 5.0.9.1 (Hall 1999), all sequences were pruned to include the complete sequences of ITS1, the 5.8S rDNA gene, and ITS2 to aid alignment. Preliminary alignments of the ITS sequences were conducted using CLUSTAL-X (Thompson et al. 1997), with manual adjustment using BioEdit for visual improvement where necessary.

Table 1. Sequences used in the phylogenetic analysis

Species	GenBank	Reference
Cadophora gregata	AY249070	Harrington & McNew 2003
	AY249071	Harrington & McNew 2003
	U66727	Chen et al. 1996
	U66728	Chen et al. 1996
	U66729	Chen et al. 1996
Cadophora malorum	DQ317328	Arenz et al. 2006
	AF083201	McKemy et al. 2005
	AF083202	McKemy et al. 2005
Phialophora americana	EU514694	Untereiner et al. 2008
	EU514695	Untereiner et al. 2008
	AF050259	Untereiner & Naveau 1999
	AF050260	Untereiner & Naveau 1999
Phialophora europaea	FJ489612	Li et al. 2008
	EF551553	Zeng & Hoog 2008
	EU514698	Untereiner et al. 2008
Phialophora sessilis	AY857542	Prenafeta-Boldu et al. 2006
	AY857541	Prenafeta-Boldu et al. 2006
	DQ363414	Feldmann et al. 2006
	FJ438386	Diaz et al. 2010
Phialophora verrucosa	DQ404353	Prodi et al. 2008
	EU514701	Untereiner et al. 2008
	AF050282	Untereiner & Naveau 1999
	AF050281	Untereiner & Naveau 1999
Pyrenopeziza revincta	AJ430224	Vralstad et al. 2002
ZJ81-D5	GU981734	This paper
ZJ81-D7	GU981735	This paper
ZJ88-B3	GU981736	This paper
ZJ88-B8	GU981737	This paper

Maximum parsimony (MP) analysis was carried out using PAUP 4.0b10 (Swofford 2001). Heuristic searches were conducted with a 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Measures calculated for parsimony included tree length, consistency index, retention index, and rescaled consistency index (TL, CI, RI and RC, respectively). To assess the robustness of clades and internal branches, a strict consensus of the most parsimonious trees was generated and a bootstrap analysis of 1000 replications was performed. The outgroup was *Pyrenopeziza revincta*.

Results

DNA phylogeny

Four isolates (ZJ81-D5, ZJ81-D7, ZJ88-B3, ZJ88-B8) were obtained from bamboo. The sequences were deposited in GenBank (ZJ81-D5 = GU981734, ZJ81-D7 = GU981735, ZJ88-B3 = GU981736, ZJ88-B8 = GU981737). Sequences for the ribosomal DNA ITS region (ITS1, 5.8S rDNA gene, ITS2) for each isolate, and related sequence data from GenBank, were used to construct a strict consensus tree with tree length (TL) = 431, consistency index (CI) = 0.8770, retention index (RI) = 0.9671, and rescaled consistency index (RC) = 0.8482 (Fig. 1). Two major clades were resolved in the MP trees. One clade, with 100% bootstrap support, contained two species, *Cadophora gregata* and *C. malorum*. The other major clade (100% bootstrap) consisted of four subclades containing isolates of *P. sessilis*, *P. europaea*, *P. americana,* and *P. verrucosa*. Our four isolates — ZJ81-D5, ZJ81-D7, ZJ88-B3, ZJ88-B8 — clustered together with *P. sessilis* with a 100% bootstrap value, indicating that they probably represent the same species.

Taxonomy

Description (Fig. 2): Hyphae initially somewhat torulose, later regularly tubular. Expanding hyphae 1.2–2.7 µm wide, smooth-walled; hyphal cells sometimes inflated to 3.8–7.5 µm wide. Phialides mostly intercalary. Collarettes distinct from the rest of the phialide, mostly sessile on undifferentiated hyphae, scattered and independent from placement of septa, triangular to funnel-shaped, up to 1.5 µm long and about 1.5 µm wide at the often somewhat flaring opening. Conidia subhyaline, smooth-walled, obovoidal to ellipsoidal, 3.0–7.3 × 2.0–4.5 µm. Conidia in slimy heads, 5.3–8.2 × 6.5–8.4 µm. Chlamydospores absent.

Specimens examined: On *Phyllostachys meyeri* McClure (*Gramineae*): China, Hubei, Xianning, Qianshan National Forest Park, 29°48'N 114°96'E, alt. 46 m, 16 Oct. 2008, J.L. Zhuang & H.L. Yang, HMUABO 20581 (with dried culture), culture ZJ81-D5 and ZJ81-

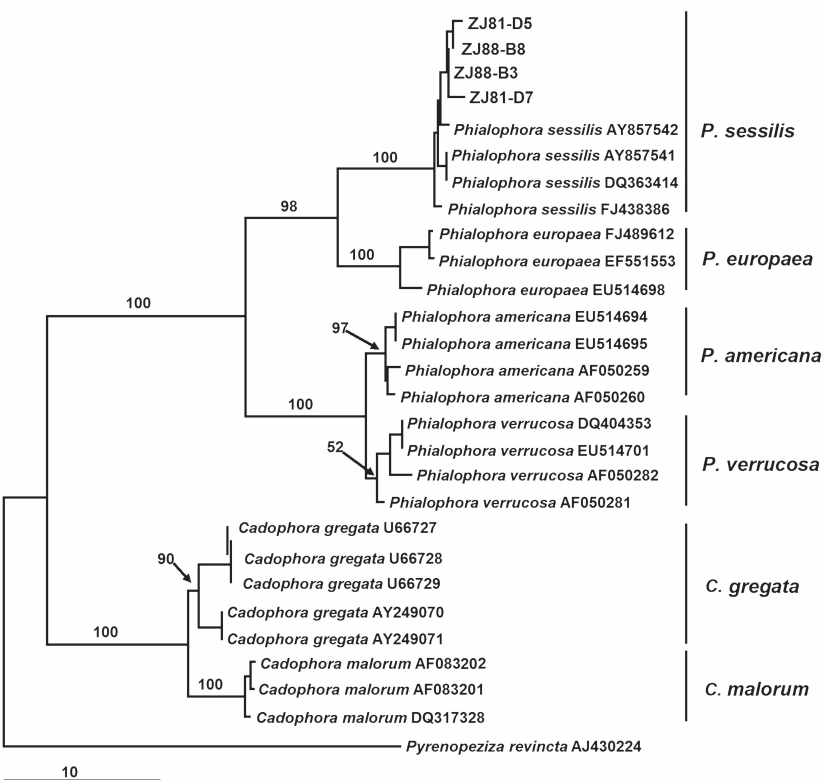

Fig. 1 The parsimony tree (TL = 431, CI = 0.8770, RI = 0.9671, RC = 0.8482) derived from a heuristic search option in PAUP version 4.0b10 with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above or below the tree branches. The tree was rooted to *Pyrenopeziza revincta*.

D7; on *Yushania falcatiaurita* Hsueh & T.P. Yi (*Gramineae*): China, Hubei, Xianning, Qianshan National Forest Park, 29°48'N 114°96'E, alt. 53 m, 16 Oct. 2008, J.L. Zhuang & H.L. Yang, HMUABO 20588 (with dried culture), culture ZJ88-B3 and ZJ88-B8.

Cultural characteristics: Colony diameter after 1 month on PDA at 22 ± 1°C reached 20 mm with even margins and rough, farinose aerial hyphae; colony centers were purplish gray and outer zones olivaceous black. On OA similar, colony reaching 23 mm diameter, flat, spreading, with sparse aerial mycelium, surface olivaceous black, but colony color lighter.

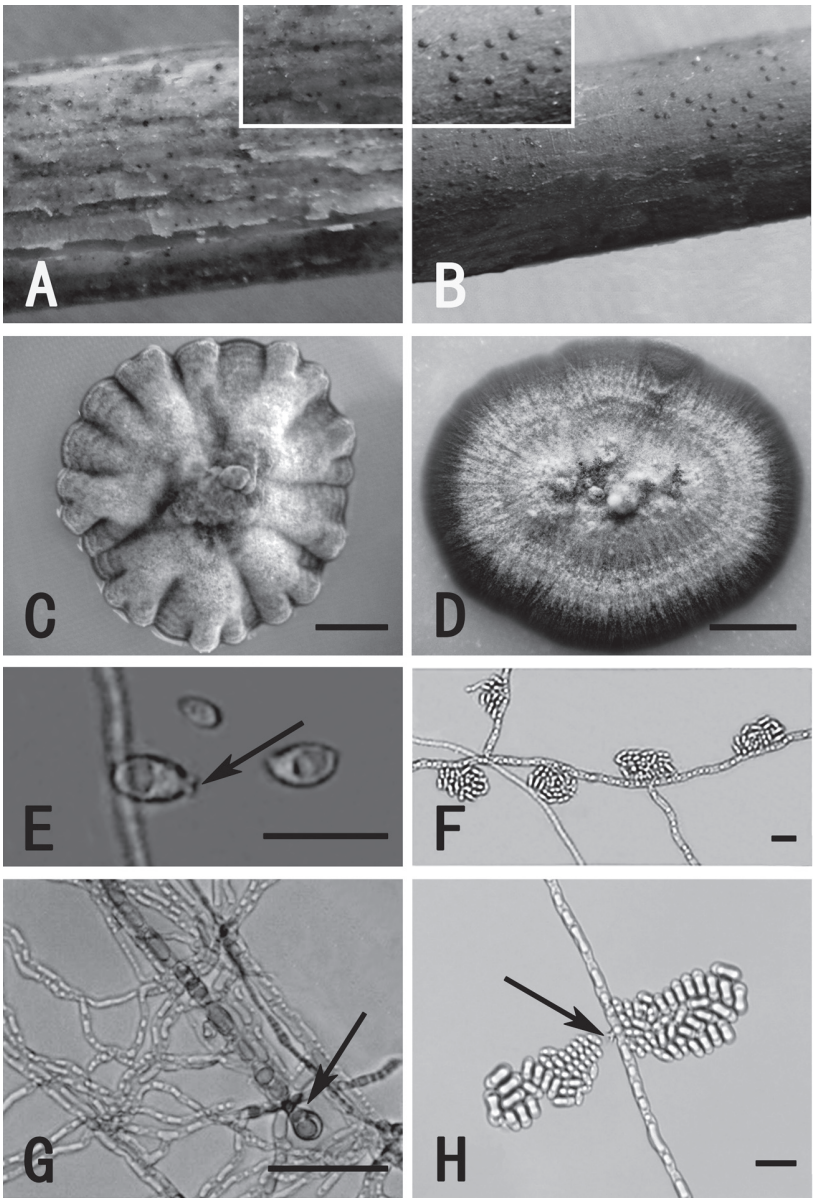

Fig. 2 *Phialophora sessilis* (ZJ81-D5). A. Signs on *Yushania falcatiaurita* with close-up view. B. Signs on *Phyllostachys meyeri* with close-up view. C. Colony on PDA after 30 days. D. Colony on OA after 30 days. E. Conidia with open collarette (arrow). F. Conidia and hyphae. G. Inflated hyphal cells (arrow). H. Phialide (arrow) bearing conidia. Bars (C–D) = 0.5 cm. Bars (E–H) = 10 μm.

Host characteristics: On *Yushania falcatiaurita*, no visible mycelial mat with shiny, black, flattened sclerotium-like bodies, round to irregular (130–470 μm diam), scattered distribution over the entire surface of the culm, densely arranged 3–8/mm^2 (Fig. 2A). On *Phyllostachys meyeri* similar, but sclerotium-like bodies were larger (430–680 μm diam), sparse, gathered in clusters on the culm, densely arranged 0.5–1.5/mm^2 (Fig. 2B). The flyspeck on bamboo did not damage the plants, but greatly reduced their ornamental and retail value. As a result, these fungi can cause significant economic losses to producers of these plants.

Discussion

Our four isolates are morphologically similar to *Phialophora sessilis* de Hoog, and despite minor differences, identity with this species was well supported by the ITS data. The same fungus caused somewhat different signs on each hosts. This phenomenon occurs in other so-called flyspeck species as well, for example, *Dissoconium mali* produced colonies with flyspeck morphology on persimmon fruit (Sun et al. 2008), but colonies with sooty blotch morphology (dark mycelial matrix) on apple fruit (Zhang 2007). It is possible that host-based morphological plasticity may also occur in other fungi in the sooty blotch and flyspeck complex.

Based on the ITS sequence analysis and morphological comparison, we identified the four isolates as *Phialophora sessilis,* which represents a new record for China. This study is also the first report of *P. sessilis* from bamboo.

Acknowledgments

This work was supported by National Natural Science Foundation of China (30771735 and Key Project No. 30630054), Program for Changjiang Scholars and Innovative Research Team in University (IRT0748), the 111 Project from Education Ministry of China (B07049) and Top Talent Project of Northwest A&F University. The authors wish to thank Dr Eric HC McKenzie (Landcare Research, Private Bag 92170, Auckland, New Zealand) and Professor Zhongyi Zhang (College of Plant Protection, Yunnan Agricultural University, Kunming, Yunnan, China) for reviewing the manuscript.

Literature cited

Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA. 2006. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biology & Biochemistry 38: 3057–3064. doi:10.1016/j.soilbio.2006.01.016

Barnes I, Gaur A, Burgess T, Roux J, Wingfield BD, Wingfield MJ. 2001. Microsatellite markers reflect intra-specific relationships between isolates of the vascular wilt pathogen, *Ceratocystis fimbriata*. Molecular Plant Pathology 2: 319–325. doi:10.1046/j.1464-6722.2001.00080.x

Batzer JC, Gleason ML, Harrington TC, Tiffany LH. 2005. Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. Mycologia 97: 1268–1286. doi:10.1094/Phyto-86-1140

Caretta G, Tosi S, Piontelli E, Hoog GS de. 2006. *Phialophora sessilis*, a lithobiont fungus. Mycotaxon 95: 281–284.

Chen W, Gray LE, Grau CR. 1996. Molecular differentiation of fungi associated with brown stem rot and detection of *Phialophora gregata* in resistant and susceptible soybean cultivars. Phytopathology 86: 1140–1148. doi:10.1094/Phyto-86-1140

Diaz MM, Batzer JC, Wang A, Harrington TC, Sutton TB, Travis JW, Cooley DR, Rosenberger DA, Wheeler M, Hartman JR, Bost SC, Yoder KS, Ellis MA, Sundin GW, Gleason ML. 2010. Diversity and biogeography of the sooty blotch and flyspeck fungi on apples in the eastern and midwestern United States. Phytopathology 100: 345–355. doi:10.1094/ PHYTO-100-4-0345

Feldmann T, Batzer JC, Gleason ML, Noga G, Oertel B. 2006. http://www.ncbi.nlm.nih.gov/nuccore/87042281?report=genbank

Gams W. 2000. *Phialophora* and some similar morphologically little-differentiated anamorphs of divergent ascomycetes. Studies in Mycology 45: 187–199.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Haase G, Sonntag L, Melzer-Krick B, Hoog GS de. 1999. Phylogenetic inference by SSU-gene analysis of members of the *Herpotrichiellaceae* with special reference to human pathogenic species. Studies in Mycology 43: 80–97.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Harrington TC, McNew DL. 2003. Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the *Helotiales*. Mycotaxon 87: 141–151.

Hoog GS de, Weenink XO, Gerrits van den Ende AHG. 1999. Taxonomy of the *Phialophora verrucosa* complex with the description of two new species. Studies in Mycology 43: 107–122.

Hoog GS de, Mayser P, Haase G, Horré R, Horrevorts AM. 2000. A new species, *Phialophora europaea*, causing superficial infection in humans. Mycoses 43: 409–416.

Kirk PM, Cannon PF, David JC, Stalpers JA. (eds). 2008. Dictionary of the fungi. 10th ed. Wallingford, CAB International.

Li DM, Hoog GS de, Lindhardt Saunte DM, Gerrits van den Ende AHG, Chen XR. 2008. *Coniosporium epidermidis* sp. nov., a new species from human skin. Studies in Mycology 61: 131–136. doi:10.3114/sim.2008.61.13

McKemy JM, Rogers SO, Wang CJK. 2005. http://www.ncbi.nlm.nih.gov/nuccore /9211040?report=genbank and /9211041?report=genbank

Medlar EM. 1915. A new fungus, *Phialophora verrucosa*, pathogenic for man. Mycologia 7: 200–203. doi:10.2307/3753363

Prenafeta-Boldu FX, Summerbell R, Hoog GS de. 2006. Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? FEMS Microbiology Reviews 30: 109–130. doi:10.1111/j.1574-6976.2005.00007.x

Prodi A, Sandalo S, Tonti S, Nipoti P, Pisi A. 2008. *Phialophora*-like fungi associated with kiwifruit elephantiasis. Journal of Plant Pathology 90: 487–494.

Sun GY, Zhang R, Zhang Z, Zhang M. 2003. Isolation of sooty blotch and flyspeck fungi from apple surface by picking up the thalli. Acta Phytopathologica Sinica 33: 479–480 [in Chinese].

Sun GY, Zhang R, Li HY, Zhang M, Gleason ML. 2008. First report of *Dissoconium mali* associated with flyspeck signs on persimmon. Phytopathology 98: S153.

Swofford DL. 2001. PAUP*. Phylogenetic analysis using parsimony (*and other methods) version 4.0 beta version. Sinauer Associates, Sunderland, Massachusetts, USA.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. doi:10.1093/nar/25.24.4876

Untereiner WA, Naveau FA. 1999. Molecular systematics of the *Herpotrichiellaceae* with an assessment of the phylogenetic positions of *Exophiala dermatitidis* and *Phialophora americana*. Mycologia 91: 67–83. doi:10.2307/3761194

Untereiner WA, Angus A, Orr MJ, Reblova M. 2008. Systematics of the *Phialophora verrucosa* complex: new insights from analyses of β-tubulin, large subunit nuclear rDNA and ITS sequences. Botany 86: 742–750. doi:10.1139/B08-057

Vralstad T, Myhre E, Schumacher T. 2002. Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the *Helotiales* in burnt and metal polluted habitats. New Phytologist 155: 131–148. doi:10.1046/j.1469-8137.2002.00444.x

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. 315–322, In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications. Academic, New York.

Zeng JS, Hoog GS de. 2008. *Exophiala spinifera* and its allies: diagnostics from morphology to DNA barcoding. Medical Mycology 46: 193–208. doi:10.1080/13693780701799217

Zhang Z. 2007. Pathogen diversity of apple sooty blotch and flyspeck. M.S. Thesis, Northwest A & F University [in Chinese].